FK409, A NOVEL VASODILATOR ISOLATED FROM THE ACID-TREATED FERMENTATION BROTH OF *STREPTOMYCES GRISEOSPOREUS*

II. STRUCTURE OF FK409 AND ITS PRECURSOR FR-900411

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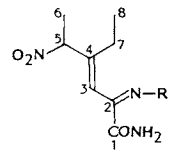
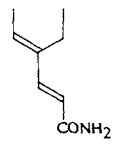
In a purification study of novel vasodilator FK409 from the fermentation broth of *Streptomyces griseosporeus*, we found the existence of FR-900411, a precursor of FK409 in the broth, suggesting that FK409 was derived from FR-900411 *via* a chemical reaction. We therefore isolated FR-900411 from the broth.

The structure of FK409 and FR-900411 were determined to be **1** and **2** respectively, on the basis of spectroscopic and chemical evidence.

As we described in the preceding paper[1], we isolated FK409 only after the acid-treatment of the fermentation broth of *Streptomyces griseosporeus* No. 16917. We therefore suggest that FK409 was generated by a chemical reaction involving a precursor in the fermentation broth. Consequently we have tried to isolate the precursor of FK409. In this paper, we describe the isolation of the precursor, FR-900411 and the structural elucidation of FK409 (**1**) and FR-900411 (**2**).



FK409 (**1**) R = OH
3 R = OCH_3



FR-900411 (**2**)

Results and Discussion

Isolation of FR-900411, the Precursor of FK409

The cultured filtrate (160 liters) of *S. griseosporeus* No. 16917 was concentrated to a volume of 16 liters under reduced pressure at pH 6.0~7.0. The concentrated solution was extracted twice with 16 liters of ethyl acetate, and the extracts concentrated under reduced pressure. The resultant oily materials (10.3 g) were applied to a column of silica gel (1,200 ml). The column was washed with a mixture of *n*-hexane and ethyl acetate (1 : 1) and eluted with a mixture of *n*-hexane and ethyl acetate (1 : 4). An amount of FR-900411 in each fraction was assayed for vasodilating activity after conversion to the active form. The method of the conversion and the assay are described below. The fractions containing the objective compound were concentrated under reduced pressure and applied to a column of silica gel (300 ml), which was developed with a mixture of chloroform and methanol (25 : 1). The objective fractions were combined and concentrated to give an oily material (FR-900411, 248 mg). Recrystallization from ethyl ether gave pure FR-900411

(110 mg) as colorless prisms.

Biological Properties of FR-900411

The relaxation activity on rat aorta and anti-platelet aggregation activity were tested. FR-900411 did not show any activity on the assay.

Structure of FK409 (1)

FK409 (**1**) is an acidic, optically inactive substance: MP 140°C (dec). The molecular formula ($C_8H_{13}N_3O_4$) of **1** was established by elemental analysis and high-resolution electron impact (HREI)-MS.

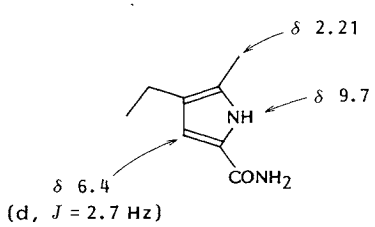
The ^{1}H NMR spectrum of **1** in acetone-d_6 showed the presence of an ethyl group (δ 1.02 (3H, t, J=7 Hz) and 2.22 (2H, q, J=7 Hz)), a unit $>CHCH_3$ (δ 1.72 (3H, d, J=7 Hz) and 5.37 (1H, q, J=7 Hz)) and an olefinic proton (δ 6.20 (1H, s)). The spectrum also exhibited the presence of three exchangeable protons (δ 11.17 (1H, s), 7.09 (1H, br s) and 6.63 (1H, br s)).

Functional groups attributable to three nitrogen atoms were deduced as follows. The IR absorption band at 1660 cm^{-1} and the ^{13}C NMR carbon signal at δ 168.1 (s) together with the exchangeable proton signal at δ 7.09 and 6.63 suggested the presence of an amide group ($CONH_2$). The IR absorption band at 1560 cm^{-1} also suggested the presence of a nitro function. Methylation of **1** with CH_2N_2 in MeOH gave the methyl derivative (**3**) (δ 4.00 (3H, s) in the ^{1}H NMR spectrum and m/z 229 (M^+) in the MS), indicating the presence of an acidic hydroxyl group which was observed as an exchangeable proton signal at δ 11.17 (1H, br s) in the ^{1}H NMR spectrum. Considering the molecular formula of **1**, this hydroxyl group should be attributed to an oxime function.

Ozonolysis of **1** gave the nitro-keto derivative **7** whose structure was established by analysis of its spectral data and comparison with the sample prepared by aldol condensation of nitroethane and propionaldehyde followed by Jones oxidation according to the method described in the literature[2]. Taking into consideration that the tri-substituted olefin, oxime and carboxamide functions are in **1** as described above, we proposed the structure **1** (without stereochemistry) for FK409.

The presumed structure **1** was further corroborated by the following transformations. Treatment of **3** with aqueous NaOH in MeOH gave rise to a product which displayed a UV absorption at 280 nm (ε 13,000), suggesting the presence of a pyrrole moiety in the molecule. Furthermore, the absence of nitro stretching bands in the IR and the establishment of the molecular formula as $C_9H_{14}N_2O_2$ (elemental analysis, MS) identified this product as **4**. This product displayed an IR adsorption at 1650 cm^{-1} ($CONH_2$), carbon signals at δ 164.0 (s, $CONH_2$), 128.2 (s, C-2), 120.0 (s, C-5), 119.4 (s, C-4), 111.7 (d, C-3), 67.1 (q, OCH_3), 20.0 (t, CH_3CH_2), 15.3 (q, CH_3CH_2) and 8.3 (q, CH_3) and proton signals at δ 6.64 (1H, s, 3-H), 6.00 (2H, br s, $CONH_2$), 3.98 (3H, s, OCH_3), 2.38 (2H, q, J=7 Hz, CH_3CH_2), 2.22 (3H, s, CH_3) and

Fig. 1. ^{1}H NMR chemical shifts for compounds **5** and **6**.



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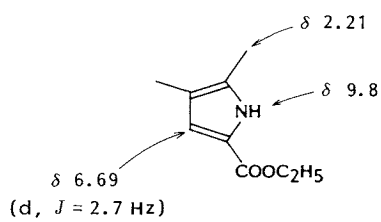

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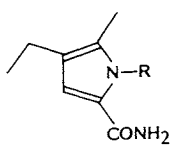
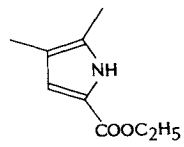


4 R=OCH_3
5 R=H



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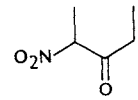

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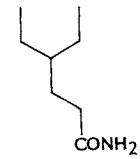

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1.14 (3H, t, J=7 Hz, CH_3CH_2). These data are all consistent with the structure **4**.

The presence of the pyrrole skeleton in **4** was further confirmed by the following reactions. Hydrogenation of **4** with Pd-C gave compound **5** which showed a positive Ehrlich reaction. Comparison of the ^{1}H NMR spectrum of **5** with that of the structurally related compound **6**[3,4] revealed that the chemical shifts and the coupling constants were superimposable as shown in Fig. 1. The cyclization of **3** to **4** suggested that the methyl derivative has the structure **3** and hence FK409 has **1**.

The geometry of the C-3–C-4 double bond was resolved by the following nuclear Overhauser effect (NOE). Upon irradiation of the 5-H and 6-H, respectively, the intensity of the 3-H signal was enhanced (+8% and +9%, respectively), while no NOE-enhancement was observed upon irradiation of any protons of the C-4-ethyl group. These facts suggested that this olefinic linkage is oriented in *E*-configuration. The structure **1** was thus proposed for FK409 except the geometry of the oxime group.

Structure of FR-900411 (2)

FR-900411 (**2**) (mp 57~58°C) is the precursor of FK409 (**1**) isolated from the fermentation broth. Elemental analysis and MS established the molecular formula of **2** as $C_8H_{13}NO$. Absorption bands at 1670, 1620 and 1590 cm^{-1} in its IR spectrum and at 260 nm in its UV spectrum suggested the presence of the conjugated diene-carboxamide moiety. The observation of α,β-olefinic proton signals at δ 7.17 (1H, d, J=16 Hz) and 5.83 (1H, d, J=16 Hz) in the ^{1}H NMR spectrum also supported the conjugated amide system. In addition the ^{1}H NMR spectrum of **2** in $CDCl_3$ showed the presence of an ethyl group adjacent to a double bond (δ 1.00 (3H, t, J=7 Hz) and 2.27 (2H, q, J=7 Hz)) and a methyl group (δ 1.78 (3H, d, J=7 Hz)) which attached to the double bond carbon bearing one hydrogen (δ 5.89 (1H, q, J=7 Hz)).

Catalytic hydrogenation of **2** over 10% Pd-C afforded the hexanamide derivative (**8**) which was identical in all respects with an authentic sample[5].

A large coupling constant (J=16 Hz) between two olefinic proton signals due to the 2-H and 3-H indicated *E*-orientation of this olefinic linkage. It was further determined that the geometry of the C-4–C-5 double bond is oriented in *E*-configuration from the synthesis of **2** as being reported in the foregoing paper. From these results the structure of FR-900411 was thus deduced to be **2**.

Experimental

IR spectra were recorded with a Jasco IRA-2 spectrometer. ^{1}H NMR spectra were measured on either a Jeol PMX-60 or a Jeol PS-100. The chemical shifts are given in ppm (δ) relative to an internal TMS standard, coupling constants (J) are in Hz and multiplicities are indicated by the usual symbols. UV spectra were measured on a Hitachi 220 A double beam spectrophotometer, absorption maxima are given in nm (extinction ε). Field desorption (FD)-MS was recorded using a Jeol JMS-D-300 mass spectrometer. MP's were measured with a Yanagimoto microscope hot-stage apparatus and are uncorrected.

FK409 (1) and FR-900411 (2)

Fermentation and isolation are described in a preceding paper[1]. FK409 (**1**) was recrystallized from MeOH: MP 140°C (dec); UV λ_{max}^{MeOH} nm (ε) 240 (sh, 7,000); IR (Nujol) cm^{-1} 3500, 3300~3200, 1660, 1600, 1560; ^1H NMR (acetone-d_6) δ 11.17 (1H, br s), 7.09 (1H, br s), 6.63 (1H, br s), 6.20 (1H, s), 5.37 (1H, q, J=7 Hz), 2.22 (2H, q, J=7 Hz), 1.72 (3H, d, J=7 Hz), 1.02 (3H, t, J=7 Hz); MS m/z 215 (M^+).

Anal Calcd for $C_8H_{13}N_3O_4$: C 44.65, H 6.09, N 19.53.
Found: C 44.64, H 5.92, N 19.60.

FR-900411 (**2**) was recrystallized from Et_2O: MP 57~58°C; UV λ_{max}^{MeOH} nm (ε) 260 (20,000); IR ($CHCl_3$) cm^{-1} 1670, 1620, 1590; ^1H NMR ($CDCl_3$) δ 7.17 (1H, d, J=16 Hz), 5.89 (1H, q, J=7 Hz), 5.83 (1H, d, J=16 Hz), 6.10~5.60 (2H, br s), 2.27 (2H, q, J=7 Hz), 1.78 (3H, d, J=7 Hz), 1.00 (3H, t, J=7 Hz); MS m/z 139 (M^+).

Anal Calcd for $C_8H_{13}NO$: C 69.03, H 9.41, N 10.06.
Found: C 67.48, H 9.12, N 9.78.

(3*E*)-4-Ethyl-2-methoxyimino-5-nitro-3-hexancarboxamide (3)

To a solution of **1** (150 mg) in MeOH (10 ml) was added excess ethereal CH_2N_2 at 0°C and the mixture was allowed to stand at 0°C for 2 hours. The excess CH_2N_2 was decomposed with acetic acid and the solvent evaporated to give a residue which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **3** (97 mg) as an oil: IR ($CHCl_3$) cm^{-1} 3540, 3400, 1690, 1550; ^1H NMR ($CDCl_3$) δ 6.56 (1H, br s), 6.08 (1H, s), 5.50 (1H, br s), 5.17 (1H, q, J=7 Hz), 4.00 (3H, s), 2.12 (2H, q, J=7 Hz), 1.74 (3H, d, J=7 Hz), 1.02 (3H, t, J=7 Hz); MS m/z 229 (M^+).

4-Ethyl-1-methoxy-5-methylpyrrole-2-carboxamide (4)

A mixture of **3** (65 mg) and aqueous 1 N NaOH (0.57 ml) in MeOH (5 ml) was stirred at room temperature for 5 minutes. The methanol was evaporated to give a residue which was poured into H_2O and extracted with $CHCl_3$. The extract was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a residue which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **4** (33 mg): UV λ_{max}^{MeOH} nm (ε) 235 (5,000), 280 (13,000); IR ($CHCl_3$) cm^{-1} 3520, 3400, 1650, 1585; ^1H NMR ($CDCl_3$) δ 6.64 (1H, s), 6.00 (2H, br s), 3.98 (3H, s), 2.38 (2H, q, J=7 Hz), 2.22 (3H, s), 1.14 (3H, t, J=7 Hz); MS m/z 182 (M^+).

4-Ethyl-5-methylpyrrole-2-carboxamide (5)

A solution of **4** (20 mg) in EtOAc (4 ml) was hydrogenated over 10% Pd-C (5 mg) at 1 atm at room temperature for 2 hours. The catalyst was then removed by filtration, and washed with EtOAc. The combined filtrate and washings were evaporated to give a residue which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **5** (15 mg): UV λ_{max}^{MeOH} nm (ε) 282 (13,000); IR ($CHCl_3$) cm^{-1} 3450, 3400, 1650, 1590, 1570; ^1H NMR ($CDCl_3$) δ 9.68 (1H, br s), 6.40 (1H, d, J=2 Hz), 5.70 (2H, br s), 2.38 (2H, q, J=7 Hz), 2.20 (3H, s), 1.10 (3H, t, J=7 Hz); MS m/z 152 (M^+).

2-Nitropentan-3-one (7)

O_3 was bubbled into a stirred solution of **1** (900 mg) in MeOH (50 ml) at −20°C for 30 minutes. To this mixture was added dimethylsulfide (1 ml) at −20°C and the mixture was warmed to room temperature over a period of 3 hours. The solvent was evaporated to give a residue which was diluted with water and extracted with pentane. The extract was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a residue which was chromatographed on silica gel. Elution with CH_2Cl_2 gave **7** (150 mg) which was identical in all respects with an authentic sample: IR ($CHCl_3$) cm^{-1} 2990, 1730, 1560, 1460, 1390; ^1H NMR ($CDCl_3$) δ 5.30 (1H, q, J=6 Hz), 2.62 (2H, q, J=7 Hz), 1.70 (3H, d, J=6 Hz), 1.07 (3H, t, J=7 Hz).

4-Ethylhexanecarboxamide (8)

A solution of **2** (100 mg) in MeOH (10 ml) was hydrogenated over PtO_2 (20 mg) at room temperature at 1 atm for 2 hours. The catalyst was filtered off and washed with MeOH. The combined filtrate and washings were evaporated to give a residue which was chromatographed on silica gel. Elution with $CHCl_3$

afforded **8** (82 mg) which was identical in all respects with an authentic sample: IR ($CHCl_3$) cm^{-1} 3480, 3400, 2960, 1670, 1590, 1460, 1380.

Conversion of the Sample to Active Form for the Bioassay

Each sample was dissolved in 1 ml of 10% aqueous MeOH and the pH adjusted to 3.0 with 6N HCl. To the solution, 0.1 ml of 10% $NaNO_2$ was added. The reaction mixture was kept mixing for 15 minutes at room temperature and extracted with 1 ml of ethyl acetate. The extract was concentrated to dryness and redissolved in 1 ml of MeOH, and then assayed as described previously[1].

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